Contribution of Transglutaminase on the Setting of Various Actomyosin Pastes

Alam AKM Nowsad,[†] Satoshi Kanoh, and Eiji Niwa

Faculty of Bioresources, Mie University, Tsu, Mie 514, Japan

(Received April 20, 1994)

The contribution of transglutaminase (TGase) to the setting of fish flesh paste was studied using TGase-free actomyosin (AM) from five fishes. Sarcoplasmic proteins (SP) were extracted from each fish and one part of them was treated with *N*-ethylmaleimide in order to inactivate TGase contained in them (NEM-SP). The addition of SP to the AM paste promoted the cross-linking of myosin heavy chain during its setting and increased the breaking force of resulting *suwari* gel. In the breaking force, the gel added with SP was higher than the one added with NEM-SP by 40% for Alaska pollack and sardine, 20% for common horse mackerel and flounder, and less than 10% for Spanish mackerel. From the result and our previous study, it is presumed that TGase increases the breaking force of the *suwari* gel from the fish flesh paste nearly by a few ten per cent.

Key words: surimi, *suwari*, transglutaminase, actomyosin

Most recently, we found that setting still proceeded even in the actomyosin paste, from which transglutaminase (TGase) was removed¹⁾ or even in the fish flesh paste, in which TGase was inactivated by the addition of SH reagents, such as *p*-chloromercuribenzoate (PCMB)²⁾ or *N*-ethylmaleimide (NEM).³⁾ From the findings, it was presumed that TGase was not always necessary for the occurrence of the setting of the fish flesh paste. However, TGase may still play an important role in the elasticity of *suwari* gel from the facts that the gel was considerably strengthened by the return of sarcoplasmic proteins to the Alaska pollack surimi, from which they were washed away,* and that the gel was considerably weakened by the removal of TGase¹⁾ or by the inactivation of TGase.^{2,3)} In this paper, we describe the contribution of TGase on the setting of the actomyosin paste from five fishes.

Materials and Methods

Materials

SA grade frozen surimi of Alaska pollack *Theragra chalcogramma* from Alyeska Seafoods, Inc., Seattle, WA, was used. Sardine *Sardinops melanosticta*, common horse mackerel *Trachurus japonicus*, flounder *Paralichthys olivaceus*, and Spanish mackerel *Scomberomorus niphonius* were purchased from a retail store. Fishes were used within one day of harvest except flounder which was brought to our laboratory alive.

Extraction of TGase from Fish Muscle

Dorsal and lateral muscles were minced, gently stirred with 5 volumes of 3.38 mM NaH₂PO₄–15.5 mM Na₂HPO₄ (pH 7.5) for 15 s, and centrifuged at 6,000 × *g* for 10 min. This washing was omitted in case of Alaska pollack mince. The washed mince was vigorously homogenized for 20 s

with 5 volumes of the above buffer to extract TGase and kept for 10 min at 4°C. The extract was centrifuged as above and dialyzed overnight against distilled water. The dialyzate was divided into two parts: one was immediately freeze-dehydrated at a final plate temperature of 10°C for 72 h (SP) and another was stirred for 3 h after the addition of crystalline NEM in a final concentration of 16 mM, dialyzed again overnight to remove excess NEM and freeze-dehydrated as above (NEM-SP).

Preparation of TGase-free Actomyosin

TGase-free actomyosin was prepared from the minces according to the previously procedure described.¹⁾ Briefly, a 100 g mince from each fish was washed 6 times by homogenization with a 500 ml of 3.38 mM NaH₂PO₄–15.5 mM Na₂HPO₄ (pH 7.5) and subsequent centrifugation at 6,000 × *g* for 10 min. Actomyosin was extracted for 3 h with a 500 ml of 0.8 M KCl–3.38 mM KH₂PO₄–20.5 mM Na₂HPO₄ (pH 8.0), washed after dilution-precipitation with water with a final centrifugation at 15,000 × *g* for 30 min and concentrated by dialyzing against polyethylene glycol powder #20,000 for 2 h. During concentration, occasional stirring was done to ensure a homogeneous removal of water from actomyosin. After concentration, the final water content of actomyosins was adjusted to 86%, the same water content as that of the FF pastes.

Preparation of Suwari Gel and Its Physico-Chemical Tests

Four types of the pastes were prepared, the pastes of fish flesh (FF), TGase-free actomyosin (AM), AM added with SP (AM + SP), and AM added with NEM-SP (AM + NEM-SP). In case of the FF pastes, the mince of the unwashed Alaska pollack surimi and that of the other fish flesh washed once as above were, respectively, ground in a mortar

[†] Present address: Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.

* K. Arai and N. Seki: Strengthening of fish gel by mixing different species of fish flesh: Factors to promote the texturization of fish paste, in "The Year Showa 63, Technology for the Utilization of Effective Nutrient Components in Fish," Research Div., Research Dept., Fisheries Agency, Japan, 1989, pp. 463–471 (in Japanese).

together with 3% NaCl (in weight of FF), 1% of sterilizer (2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (0.025%), sorbic acid (20%), and glyceryl ester of fatty acid (5%) in starch, a former type of Solmighy, Ueno Fine Chem. Co.), and distilled water at 4°C for 10 min. In case of the AM, AM + SP, and AM + NEM-SP pastes, AM was ground with and without SP or NEM-SP besides that of the same amount of NaCl and sterilizer as in the case of FF paste. The amount of SP added was 16% in the wet weight of AM for Alaska pollack, 10% for sardine and common horse mackerel, 12% for flounder, and 7% for Spanish mackerel, so that their TGase activity raised up to the same level as in the FF pastes as shown in Table 1 later. The amount of NEM-SP added was the same in weight as that of SP. The resulting pastes were stuffed into glass tubes (10 mm inner diameter, 45 mm length), set in a water bath after wrapping both ends of the tube with parafilm and cooled in ice-water. Setting temperature was varied suitable for each fish (30°C for Alaska pollack and sardine, 35°C for flounder, and 40°C for common horse mackerel, and Spanish mackerel).

Puncture test was done by using a rheometer (Fudoh Kogyo Co., NRM 2010J) with a spherical plunger (5 mm diameter) at a table speed of 6 cm/min on the gels sliced into 15 mm height.

The pH of the pastes and gels was measured by pressing a small amount of sample on a hollow chamber of an electrode (Horiba Ltd., Twin Compact pH meter, B-112).²⁾

TGase Activity Measurement

TGase activity was measured according to the method of Wan *et al.*,⁴⁾ with a few modifications⁵⁾ and expressed as the amount of monodansyl cadaverine (MDC) incorporated into the muscular proteins after 7 h of incubation of the pastes at the temperatures adopted for their setting.

SDS-PAGE

The *suwari* gels were subjected to SDS-PAGE (5% acrylamide gel) and the relative amount of protein subunits in the electrophoresed disks were obtained densitometrically as reported previously.¹⁾

Results and Discussion

It was confirmed by Shimizu *et al.*⁶⁾ that the setting of the fish flesh paste proceeds more easily in the order of Alaska pollack, sardine, flounder, common horse mackerel, and Spanish mackerel. In this study, the setting temperature was heightened in the hard-setting fishes, so as to promote the setting effectively. The pH of the FF and AM pastes ranged from 6.6 to 6.8 and scarcely changed by the addition of SP or NEM-SP, suggesting that the physico-chemical properties of the paste were not affected by the pH change upon the addition of SP or NEM-SP.

Table 1 shows the TGase activity of the FF pastes, AM ones, AM + SP ones, and AM + NEM-SP ones measured at the temperatures adopted in the setting of each paste for 7 h, the longest setting time taken in this study. The TGase activity of the FF pastes was high in the order of Alaska pollack, sardine, flounder, common horse mackerel, and Spanish mackerel, the same as the order of the easiness of the setting. From the result that the activity was zero in all

Table 1. TGase activity in the various pastes

Pastes	Fishes				
	Alaska pollack	Sardine	Common horse mackerel	Flounder	Spanish mackerel
	Incubation temperature (°C)* ¹				
	30	30	40	35	40
	TGase activity (nmol MDC/mg protein)* ²				
FF	3.5	2.9	2.0	2.5	1.1
AM	0.0	0.0	0.0	0.0	0.0
AM + SP	3.4	2.6	2.3	2.3	0.9
AM + NEM-SP	0.0	0.0	0.0	0.0	0.0

*¹ TGase activity was measured at the same incubation temperatures as those employed for setting.

*² The amount of MDC incorporated into the muscle proteins after 7 h of incubation.

Fig. 1. Change in CMHC and MHC in the various pastes during the setting.

—○—: CMHC; —■—: MHC.

the AM pastes, it is suggested that TGase was removed by washing the fish minces 6 times. The TGase activity was roughly the same in the AM + SP pastes and FF ones, but zero in the AM + NEM-SP ones, which shows that TGase was inactivated by the treatment with NEM.

Figure 1 shows the change in cross-linked myosin heavy chain (CMHC) and MHC during the setting of the above four types of the pastes. With the progress of the setting,

Fig. 2. Change in the breaking force of the *suwari* gels prepared from the various pastes during setting.

—●—: FF; —■—: AM; —○—: AM + SP; —□—: AM + NEM-SP.

CMHC gradually increased in the FF pastes and this increment was much in the order of the easiness of the setting. The increment was slightly less in the AM + SP pastes than in the FF pastes but considerably more than in the AM pastes. The increment in the AM + NEM-SP pastes was nearly the same level as in the AM pastes. In any paste, however, the increment was more in the easy-setting fishes. On the other hand, MHC gradually decreased with the progress of the setting, and this decrement was more in the FF and AM + SP pastes than in the AM and AM + NEM-SP pastes and more in the easy-setting fishes in any paste. This kind of behavior of CMHC and MHC suggests that the cross-linking of MHC still proceeds, especially in the easy-setting fish, even in the condition that the TGase activity is zero. MHC may be polymerized not only through covalent bonds formed by the catalysis of TGase but also through non-covalent bonds presumably formed by thermal aggregation.^{1,7,8)}

Figure 2 shows the changes in the breaking force of the *suwari* gels prepared from the above four types of the pastes.

Within 1 h of setting, the breaking force rapidly increased and slowly thereafter. The increment of the breaking force was much in the order of the FF gels, AM + SP ones, AM ones, and AM + NEM-SP ones. This increment was, however, considerably a little in the last two, and little difference could be detected between them in that increment. Furthermore, the increment was high in any gel in the order of Alaska pollack, sardine, common horse mackerel, flounder, and Spanish mackerel, nearly the same as the order of the easiness of the setting, although the setting temperature was lower in the easy-setting fishes, suggesting that the species-specificity among the fishes in the easiness of setting is kept irrespective of the action of TGase. In the breaking force, the AM + SP gels was higher than the AM + NEM-SP ones by 40% for Alaska pollack and sardine, 20% for common horse mackerel and flounder, and less than 10% for Spanish mackerel after a 7 h setting. The increment of the breaking force seems roughly to be more in the easy-setting fishes.

Considering the above results and our previous ones that the breaking force of the *suwari* gel from Alaska pollack was lowered by about 35% by the addition of PCMB²⁾ and NEM,³⁾ it is presumable that TGase increases the breaking force of the *suwari* gel from the fish flesh pastes nearly by only a slight ten per cent.

References

- 1) A. AKM. Nowsad, S. Kanoh, and E. Niwa: Setting of transglutaminase-free actomyosin paste prepared from Alaska pollack surimi. *Fisheries Sci.*, **60**, 295–297 (1994).
- 2) A. AKM. Nowsad, S. Kanoh, and E. Niwa: Setting of surimi paste in which transglutaminase is inactivated by *p*-chloromercuribenzoate. *Fisheries Sci.*, **60**, 185–188 (1994).
- 3) A. AKM. Nowsad, S. Kanoh, and E. Niwa: Setting of surimi paste in which transglutaminase is inactivated by *N*-ethylmaleimide. *Fisheries Sci.*, **60**, 189–191 (1994).
- 4) J. Wan, J. Miura, and N. Seki: Effects of monovalent cations on cross-linking of myosin in *suwari* gels from walleye pollack. *Nippon Suisan Gakkaishi*, **58**, 583–590 (1992).
- 5) A. AKM. Nowsad, S. Kanoh, and E. Niwa: Effects of amine salts on the elasticity of *suwari* gel from Alaska pollack. *Nippon Suisan Gakkaishi*, **59**, 1017–1021 (1993).
- 6) Y. Shimizu, R. Machida, and S. Takenami: Species variations in the gel forming characteristics of fish meat paste. *Nippon Suisan Gakkaishi*, **47**, 95–104 (1981).
- 7) A. AKM. Nowsad, S. Kanoh, and E. Niwa: Electrophoretic behavior of cross-linked myosin heavy chain in *suwari* gel. *Nippon Suisan Gakkaishi*, **59**, 667–671 (1993).
- 8) T. Shoji, H. Saeki, A. Wakameda, and M. Nonaka: Influence of ammonium salt on the formation of pressure-induced gel from walleye pollack surimi. *Nippon Suisan Gakkaishi*, **60**, 101–109 (1994).